

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Shipman et al.
Serial No.: to be assigned
Filed: herewith
For: Method and Reagents for Testing for Mutations in the BRCA1
Gene

PRELIMINARY AMENDMENT

Asst. Commissioner for Patents
Washington, D.C. 20231

Sir:

Preliminary to the examination of the continuation application filed herewith,
please make the following amendments:

In the specification:

Page 1, please amend the paragraph spanning lines 1-3 to read as follows:

This application is a continuation of US Patent Application Serial No. 08/649,950,
filed May 14, 1996, which is a continuation-in-part of US Patent Application Serial No.
08/271,946, filed July 8, 1994, now US Patent No. 5,545,527, both of which are incorporated
herein by reference.

In the claims

Please cancel claims 1-52 and add claims 53-65 as follows:

53. A method for testing a sample for mutations in the BRCA1 gene
comprising the steps of

- (a) amplifying two or more exons or partial exons of BRCA1 to produce amplified fragments, wherein the two or more exons or partial exons are amplified in a multiplex amplification reaction to produce multiplex amplification products for analysis;
- (b) determining the sizes and amounts of amplified fragments in the multiplex amplification products and comparing the determined sizes or amounts to standard values for amplification of the same exons or partial exons of wild-type BRCA1 gene, wherein a difference in fragment size or amount is indicative of the presence of a mutation in the BRCA1 gene; and
- (c) if no mutation is detected in the BRCA1 gene as a result of the determination of the sizes and amounts of the amplification fragments, determining the sequence of one or more exons of the BRCA1 gene.

54. The method according to claim 53, wherein at least exons 2, 11 and 20 of the BRCA1 gene, or portions thereof, are amplified in one multiplex amplification reaction.

55. The method according to claim 53, wherein exons 2, 5, 9 and 14 of the BRCA1 gene, or portions thereof, are amplified in one multiplex amplification reaction.

56. The method according to claim 53, wherein exons 3, 7 and 15 of the BRCA1 gene, or portions thereof, are amplified in one multiplex amplification reaction.

57. The method according to claim 53, wherein the sequence is determined by amplifying a selected one of the multiplex amplification products in an aliquot of the multiplex reaction mixture and then sequencing the amplified multiplex amplification products.

58. The method according to claim 57, wherein the amplification of the multiplex reaction mixture and the sequencing of the amplified multiplex reaction product are performed in a single vessel.

59. The method according to claim 57, wherein the multiplex reaction mixture is amplified by combining the multiplex reaction mixture with an amplification mixture containing two primers for the selected one of the multiplex reaction products, a mixture of dNTP's and a thermostable polymerase in a buffer suitable for amplification, and exposing the resulting combination to a first series of thermal cycles including at least an extension phase and a denaturation phase to produce an amplified mixture containing the amplified multiplex reaction product; adding a sequencing mixture comprising a chain terminating nucleoside triphosphate to the amplified mixture and exposing the resulting combination to a second series of thermal cycles including at least an extension phase and a denaturation phase to produce sequencing fragments; and evaluating the size of the sequencing fragments.

60. The method according to claim 59, wherein the thermostable polymerase is Thermo Sequenase®.

61. The method according to claim 59, wherein the sequencing mixture further comprises a fluorescently labeled sequencing primer.

62. The method according to claim 61, wherein the sequencing mixture further comprises a thermostable polymerase for sequencing which incorporates dideoxynucleosides into an extending oligonucleotide at a rate which is no less than about 0.4 times the rate of incorporation of deoxynucleosides in an amplification mixture.

63. The method according to claim 62, wherein the thermostable polymerase for sequencing is Thermo Sequenase®

64. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify at least

two different exons or portions of exons of the BRCA1 gene in a multiplex amplification reaction.

65. The kit according to claim 64, wherein the primers are selected for amplification of exons 2, 5, 9 and 14 of the BRCA1 gene in one multiplex amplification reaction.

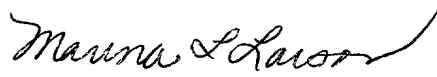
REMARKS

This preliminary amendment is presented to add a complete reference to the parent case and to a new set of claims for consideration by the Examiner. Claims 53-65 correspond to claims 1, 2, 6, 8, 19-26 and 29 in the parent case (serial no. 08/649,950). These claims were rejected in that case over combinations of references which in each case included US Patent No. 5,545,527 of Stevens et al. The inventions of this application and the Stevens patent are commonly assigned, and were the subject of a common obligation to assign at the time the inventions were made. The Examiner acknowledged in the Office Action mailed July 18, 2001 (Page 9), that filing of a continuing prosecution application together with such a statement would exclude the Stevens et al. reference as prior art. Applicants respectfully submit that the same is true for the present continuation application. Thus, it is respectfully submitted that none of the rejections made in the parent case are available, and that the claims presented in this application are in form for allowance.

VGEN.P-028-2
PATENT APPLICATION

Applicants enclose terminal disclaimers for this application over the parent case and the Stevens patent, together with the appropriate fee.

Respectfully submitted,



Marina T. Larson
Patent Office Reg. No. 32,038
Attorney for Applicant(s)
(970) 468-6600

MARKED UP COPY OF AMENDMENTS

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